A High Molecular Weight Protease in the Cytosol of Rat Liver

I. PURIFICATION, ENZYMEOLOGICAL PROPERTIES, AND TISSUE DISTRIBUTION*

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Rat liver cytosol has low hydrolytic activity against [3H]methylcasein at neutrality, but activity increases greatly on addition of various compounds such as poly-L-lysine, N-ethylmaleimide, and sodium deoxycholate, suggesting that it contains latent proteolytic activity. The latent enzyme was found to be stabilized in the presence of 20% glycerol and to be activated by addition of poly-L-lysine. The latent enzyme was purified from a crude extract of rat liver to apparent homogeneity in the presence of 20% glycerol by conventional chromatographic techniques.

The purified enzyme showed endoproteolytic activity toward various proteins when it was activated by the compounds listed above. It preferentially degraded N-substituted tripeptide substrates with a basic amino acid at the carboxyl terminus, as well as peptides containing neutral hydrophobic amino acids. It did not require activation for these peptidase activities, in contrast to its activity toward large proteins. Interestingly, a proteinase and a trypsin-like and a chymotrypsin-like peptidase activity could not be separated by customary chromatographic methods but were distinguishable by their sensitivities to various inhibitors, activators, and covalent modifiers, suggesting that the enzyme has three distinct active sites within a single protein. The enzyme seems to be a seryl endopeptidase showing maximal activity at neutral and weakly alkaline pH values. Thus, the enzyme is a unique protease with latent multifunctional catalytic sites.

The distribution of the protease is soluble extracts of various rat tissues and cells was examined quantitatively by an enzyme immunoassay. The enzyme level was highest in liver and also in spleen, stomach, lung, small intestine, and kidney, but was low in heart, diaphragm, skeletal muscle, brain, and skin. The concentrations of enzyme in some established cell lines including hepatoma and rat kidney cells were comparable to that in normal liver hepatocytes. The enzyme was found mainly in the cytosol fraction, although a small amount was associated with microsomal membranes, suggesting that it is an extralysosomal protease. Immunohistochemical staining of the liver and skeletal muscles showed that the protease is distributed diffusely in panlobular hepatocytes with slight centrolobar predominance and is present in Kupffer cells, vascular endothelial cells, and bile duct epithelial cells in the liver and also diffusely in the intermyofibrillar spaces and vascular endothelial cells in skeletal muscle. The quantitative data obtained in the present study indicate the presence of the protease in the cytosol fraction of all rat tissues. This ubiquitous distribution indicates the general importance of this enzyme, presumably in a nonlysosomal pathway of protein breakdown.

In animal cells, there are two distinct mechanisms for degradation of intracellular proteins which probably function in different biological settings (1-3). One is a lysosomal system, which seems to play the main role in controlling the free amino acids pools necessary for adaptation to changes of nutritional and hormonal status (4). This system is also involved in breakdown of endocytosed proteins in cells with phagocytic activity (3). Many proteolytic enzymes, such as cathepsins, have been shown to be components of this system, which can degrade proteins completely to their constituent amino acids. The other system is a nonlysosomal system, which appears to be involved in breakdown of abnormal proteins with highly aberrant structures that are generated in cells by mutation or errors of protein synthesis or posttranslational damage (1, 2). There is evidence that proteins with short half-lives and those that are injected into the cytosol are degraded by this system (5, 6). The concept of a nonlysosomal pathway of degradation is based on the fact that many known lysosomal inhibitors are unable to reduce the rates of degradation of these proteins and that the degradative process is not affected by amino acid starvation, which induces vacuolar catabolism in the lysosomes. Thus, a nonlysosomal route of protein degradation is clearly present in cells, presumably in the cytoplasmic compartment. Since the protease activity in the cytosol has been found to be very much lower than that in lysosomes, this route has attracted little attention. However, recently there have been many reports that mammalian cytosol contains a protease with a molecular weight of over 400,000 (7-15) that is presumably responsible for nonlysosomal proteolysis because of its cytosolic localization. Sarcely any protease activity is detectable in the crude cytosol, but strong activity is found after various treatments of the cytosol, such as DEAE-cellulose and molecular sieve chromatography. These findings suggest that the protease is present in a masked state in the cytosol or that these purification procedures remove an inhibitor or proteins that compete with the

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† To whom correspondence should be addressed.
substrate in the enzyme assay. Recently, we found a latent proteolytic enzyme in crude extracts of rat liver that was activated by the chromatographic procedures described above. Its activity could be important in regulation of the nonlysosomal route of protein breakdown. Its presence in a latent state presumably prevents autolysis of intracellular proteins.

Of particular interest is the fact that the enzyme appears to be a multifunctional protease with at least three independent catalytic sites in one molecule (11, 12). This unique property is clear evidence that it differs from other well-established proteases (3). So far, little is known about the physiological significance of this protease. One way to evaluate its role in cells is to obtain information concerning its cellular and tissue distributions. This study reports the purification of this latent endoprotease from the cytosol of rat liver to apparent homogeneity, some of its properties, and its tissue distribution in rats as measured by immunological techniques.

EXPERIMENTAL PROCEDURES AND RESULTS

As shown in the present study, rat liver cytosol contains latent proteolytic activity that can be activated by various treatments (Table I). Similar latent activity was found in the cytosol fractions of various other rat tissues tested, including skeletal muscle and kidney, suggesting that this activity is present in the cytosol of many tissues. In this work, we purified the soluble endoprotease in its latent form. The latent enzyme seems to be responsible for the masked cytosolic activity of the crude extract for the following reasons. 1) The purified enzyme exhibits latent endoproteolytic activity and may be activated by the compounds that activated the crude liver extract. 2) The pH dependences of the caseinolytic activities of the crude extract and of the purified protease in the absence and presence of poly-L-lysine are essentially similar (Figs. 1 and 9). 3) The protease in the crude cytosol is eluted as a single major fraction on DEAE-cellulose chromatography (Fig. 2). 4) It is active under neutral and weakly alkaline conditions (Fig. 9). 5) The activity of the crude extract is almost completely lost on immunoprecipitation with monospecific antibody (data not shown).

Latency may be a physiologically crucial feature of this cytosolic enzyme because if it were present in an activated form it would cause autolysis of intracellular proteins. There is, however, no evidence that the enzyme activation described in this paper is related to physiological changes in the cells. The activation is apparently not due to removal of a potential inhibitor bound to the protein since activation caused no change in the molecular weight of the enzyme as judged by gel filtration. Moreover, the activation results in no change in the secondary structure as judged by circular dichroism (data not shown). Thus, the activation may be due to unmasking of the active site of a large molecule, which would result in only a slight change in conformation. Although we found that the enzyme can be purified in its latent form in the presence of glycerol, the reason why glycerol stabilizes it is unknown. Glycerol also prevented denaturation of the helical content in the secondary structure of the enzyme by urea, temperature, and salt (data not shown). To understand the molecular structure of this high molecular weight protease, it will be interesting to determine how glycerol maintains it in a latent state.

Thus, studies on the mechanism of activation should be useful for characterizing the structure of the enzyme. Subsequent physicochemical studies (30) showed that the protease is a large molecule ($M_c = 720,000-760,000$) and could not be dissociated into smaller components by the usual methods. Although the purified enzyme gave a single protein band on nondenaturing PAGE, on SDS-PAGE it gave several protein bands. The appearance of these multiple components has been observed by others (11, 12, 15). At present, it is unknown whether these components are distinct subunits of a large enzyme complex or are generated by autodigestion. Although the latter possibility may be more probable because the disopropyl fluorophosphate was detected in several bands separated by SDS-PAGE, more detailed studies are necessary to clarify the subunit structure of this high molecular weight protease.

The large size of this protease may affect its latent function. Its large size may also be related to its unique catalytic functions. As shown in Table III, we found that the high molecular weight protease has a broad substrate specificity. It is important to find whether this is due to one or multiple catalytic sites. The enzyme shows three types of activity: a latent protease activity and two peptidase activities on small peptides which are substrates for trypsin and chymotrypsin. These three activities could not be separated by the usual chromatographic techniques, but were distinguishable with various inhibitors or activators. For example, leupeptin and chymostatin specifically inhibited the degradation of benzoylarginine-Ala-Arg-Arg-methoxynaphthylamine and glutaryl-Ala-Ala-Phe-methoxynaphthylamine, respectively, without affecting the activity on casein (Table IV). Moreover, the two peptidase activities were strongly inhibited by tryptic digestion (data not shown) and sulfhydryl-blocking reagents which caused marked activation of the protease activity, indicating that the active sites for proteins and peptides are different. Furthermore, covalent modifiers such as diisopropyl fluorophosphosphate and phenylmethanesulfonyl fluoride had different effects on the two activities. These findings suggest that the enzyme has three distinct active sites within one large protein. In other words, it may be regarded as a multifunctional protease complex.

Wilk and Orlowski (11) and Dahlmann et al. (12) found similar multicatalytic proteases in bovine pituitary and rat skeletal muscle, respectively. Besides trypsin-like and chymotrypsin-like activities, they observed activity on glutamyl peptide bonds. The hydrolytic activity on acidic amino acids at the carboxyl end appears to be related to the caseinolytic activity rather than to the trypsin-like and chymotrypsin-like activities. Moreover, Rivett (15) showed that the liver enzyme cleaves insulin on the carboxyl side of glutamine. Thus, the enzyme seems to act as a variety of peptide bonds involving neutral, basic, and acidic amino acids, and this broad specificity may be advantageous for rapid degradation of proteins. These multiple functions...
appear to be common properties of our liver enzyme and the enzymes described by others.

High molecular weight proteases have been found in a wide variety of mammalian tissues (7–15). These enzymes may not all be the same since they do not have exactly the same properties. However, there are some crucial differences between our enzyme and those reported by others. The major difference is that the enzymes reported by others were defined as thiol proteases from their sensitivity to sulphydryl-blocking reagents, whereas our enzyme is activated by alkylation of sulphydryl residues and the activated form is very sensitive to a sulphydryl-blocking reagent. Moreover, its activities are not affected by Ep-475 (32), a specific inhibitor of cysteine endopeptidases, suggesting that the enzyme is not a thiol protease. In addition, this enzyme is inhibited by a covalent modifier such as diisopropyl fluorophosphate and phenylmethanesulfonyl fluoride (Table IV), and [3H]diisopropyl fluorophosphate was incorporated into the purified enzyme (Fig. 8), strongly indicating that the enzyme is a serine protease. Another difference is that in skeletal muscle peptidase activities are activated by SDS (11, 12) and fatty acids (12), whereas the peptidase activities of our enzyme were present without addition of these compounds. This difference is not due to differences in enzyme sources because we have purified the same enzyme from skeletal muscle (data not shown). The difference between our enzyme and those reported by others may be in purification: we purified the enzyme in a latent form, whereas, by our definition, others purified enzymes in an activated state. One possibility is that changes in multiple conformational states affect the catalytic activity and that enzymes with different structures are prepared by different purification procedures.

In the present work, we examined the distribution of a high molecular weight protease in various rat cells and tissues. In general, it is difficult to determine the amount of a proteolytic enzyme from its activity because it is difficult to identify the specific substrate of a given protease. We therefore used a sandwich enzyme immunoassay method recently developed by Ishikawa et al. (26). This method is very sensitive and is quantitative for measuring the absolute amount of enzyme. With this method, the minimum detectable amount of the enzyme was 100 pg/assay (Fig. 11). The present results show that the high molecular weight protease is widely distributed in many rat cells and tissues. Its content per milligram of material is the more probable since the enzyme is almost completely associated with the cytosol fraction (Table VI). Its presence in this fraction is due to contamination of the fraction with cytosolic material because this fraction contained less than 1% of lactate dehydrogenase, which is a marker of the soluble fraction. One possible explanation for its presence in the microsomal fraction is that some of the enzyme may be in a microsomal-associate form. Another possibility is that it was precipitated because of its unusually large size. Moreover, its presence in this fraction is an artifact. The latter possibility is the more probable since the enzyme is almost completely precipitated by the centrifugation used to prepare the microsomal fraction for 20 h (results not shown).

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REFERENCES

Continued on next page.
**Supplementary materials to a high Molecular weight protein in the cytosol of Rat liver**

**MATERIALS AND METHODS**

**As a High Molecular Weight Protein in the Cytosol of Rat Liver**

**Preparation of Subcellular Fractions:** The liver was homogenized in 0.32 M sucrose containing 10 mM Tris-HCl buffer (pH 7.4) and recentrifuged at 105,000 g for 1 hour. The supernatant was then collected as the cytosol fraction. The cytosol was incubated with the protease inhibitor cocktail (10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml E-64, and 1 mM PMSF) for 30 minutes at 4°C.

**Assay of Proteolytic Activity:** The assay was conducted in 0.1 M sodium phosphate buffer (pH 7.4), containing 0.1 M NaCl, 0.1 mM ZnCl2, and 1 mM CaCl2. The reaction was initiated by the addition of 30 μl of the cytosolic fraction (approximately 2 mg protein) to 1 ml of the substrate solution. The substrate solution contained 0.1 M NaCl, 0.1 mM ZnCl2, 0.1 mM CaCl2, and 20 μCi of [14C]-leucine. The reaction was allowed to proceed for 30 minutes at 37°C. The reaction was stopped by the addition of 1 ml of ice-cold trichloroacetic acid (10%). After 15 minutes, the supernatant was collected and the radioactivity was determined by liquid scintillation counting. The results were expressed as units of activity per mg of protein.

**Effects of Protease Inhibitors:** The effect of various protease inhibitors on the proteolytic activity of the cytosolic fraction was determined. The cytosolic fraction was preincubated with varying concentrations of the protease inhibitors for 30 minutes at 37°C before the addition of the substrate solution. The reaction was conducted as described above.

**Results:**

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**Discussion:** The results of this study indicate that the cytosolic protease activity is inhibited by a variety of protease inhibitors, suggesting that the activity is due to a specific protease. The inhibition of protease activity by the inhibitors suggests that the activity is due to a specific protease. Further studies are required to identify the specific protease responsible for the activity.

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**References:**

Large Protease of Rat Liver Cytosol

For immunological detection of the enzyme, the specificity of the antibody prepared was evaluated by immunoelectrophoresis. As shown in Fig. 1B, the antibody was specific for the enzyme, because it gave a single precipitation line on immunoelectrophoresis against both trypsinogen and trypsin. Purified rat liver protease was also cross-reacted with the enzyme of the other rat tissues tested, as indicated by the formation of single precipitin lines and fusion of these lines in the following double diffusion tests (Fig. 1D).

Thus, enzymes with the same immunoreactivity are present in many rat tissues. The tissue content of the enzyme was determined by an enzyme immunoassay method using a solid phase with immobilized rat liver protease and rabbit-protasien antibodies. This sandwich method was very sensitive and quantitative for detecting the antigen of the enzyme in crude extracts. A typical standard curve of the enzyme from rat liver is shown as a typical presentation of the calibration curve (Fig. 2).

The enzyme activity of rat liver cytosol was determined by the optimal sample volume of each extract for assay. Results showed that the amounts of protease were proportional to the volumes of extracts employed in the immunosorbent under the respective conditions. Thus, appropriately diluted extracts were used for immunoeassay of the enzyme.

The concentrations of the protease in soluble extracts of various rat tissues are shown in Table 3 (upper column). The highest concentrations of the protease per g wet tissue were found in liver, followed by those in adrenal glands, brain, and lung. In contrast, the lowest concentrations were found in spleen, kidneys, eyes, heart, and skin. In some cases, the enzyme was not detected in peripheral tissues, such as skin and brain. These results are in good agreement with the results of immunoelectrophoresis and immunofluorescence staining of the tissues. The enzyme in liver and brain was not affected by cryopreservation or freezing of the tissue in liquid nitrogen, and its concentration was not altered by exposure to different temperatures or pH conditions. Therefore, the enzyme is present in many rats and can be detected in the cytosolic fraction of the liver, heart, and brain.

In this study, the effects of protease inhibitors on the activity of the enzyme were determined. The inhibitor of the enzyme was obtained from the serum of rat liver cytosol and found to be effective in inhibiting the activity of the enzyme. The enzyme activity of the cell-free extract of rat liver cytosol was determined by the assay described above. The enzyme activity was measured at various concentrations of the inhibitor and found to be inhibited in a dose-dependent manner. The enzyme activity was also inhibited by the addition of the peptide substrate, but not by the ribonuclease or phosphatase substrate.

Finally, the effect of protease inhibitors on the activity of the enzyme was determined using the assay system described above. The enzyme activity was measured in the presence and absence of protease inhibitors, and the results are shown in Fig. 2. The enzyme activity was inhibited by the addition of the protease inhibitors, but not by the control buffer.

Fig. 3: Hydroxyapatite chromatography. The details of the procedure were described in the text. Symbols are as for Fig. 1. Removal of phosphate by dialysis was essential for the enzyme assay, because phosphate strongly inhibited the protease activity in the absence of glycerol.

Fig. 4: Sepharose 4B-Ca study chromatography. Details of the procedure were described in the text. Symbols are as for Fig. 1.
Large Protease of Rat Liver Cytosol

Fig. 1 illustrates the caseinolytic activity of various compounds on rat liver cytosol. The effects of different compounds on the caseinolytic activity are shown in Table I.

Table I: Effects of Various Compounds on the Caseinolytic Activity of Rat Liver Cytosol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3H Casein Hydrolysis (μg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9</td>
</tr>
<tr>
<td>Poly-L-lysine</td>
<td>10.3</td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>4.8</td>
</tr>
<tr>
<td>SDS</td>
<td>6.7</td>
</tr>
<tr>
<td>Urea</td>
<td>3.3</td>
</tr>
<tr>
<td>Freezing</td>
<td>28°C, 3 weeks, 7.8</td>
</tr>
<tr>
<td>Heating</td>
<td>55°C, 3 min, 3.6</td>
</tr>
</tbody>
</table>

Crude extracts were prepared as described in Experimental Procedures and samples of 0.2 mg of protein were used for assays. Data are typical for several independent experiments, which gave similar results, but showed different magnitudes of stimulation.

Table II: Purification of High Molecular Weight Protease from Rat Liver

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Protein (μg)</th>
<th>3H Casein Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Extract</td>
<td>35240</td>
<td>59</td>
</tr>
<tr>
<td>DEAE-Cellulose</td>
<td>3751</td>
<td>242</td>
</tr>
<tr>
<td>Polyethylene Glycol Fraction (U)</td>
<td>2554</td>
<td>12</td>
</tr>
<tr>
<td>Biogel A-1.5m</td>
<td>81</td>
<td>141</td>
</tr>
<tr>
<td>Heparin-Sepharose CL-4B</td>
<td>111</td>
<td>6</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>65</td>
<td>92</td>
</tr>
<tr>
<td>DEAE Affi-Gel Blue</td>
<td>48</td>
<td>58</td>
</tr>
</tbody>
</table>

The enzyme was purified from 650 g of liver from 10 rats. Values are typical for ten separate purifications.

Fig. 11 shows the calibration curve for the caseinolytic activity of a high molecular weight protease from rat liver. Points are means for duplicate determinations. Similar results were obtained in individual assays in 10 or more separate experiments.
Table I

Substrate Specificity of the Purified Enzyme

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (mM)</td>
<td>%</td>
</tr>
<tr>
<td>1% Labeled Protein Substrate</td>
<td></td>
</tr>
<tr>
<td>A-Casein</td>
<td>15.1</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>15.6</td>
</tr>
<tr>
<td>Deaminated A-Casein</td>
<td>27.3</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11.9</td>
</tr>
<tr>
<td>Deaminated hemoglobin</td>
<td>7.6</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>16.7</td>
</tr>
<tr>
<td>Deaminated Immunoglobulin</td>
<td>1.7</td>
</tr>
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Symmetrical Peptide Substrate

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (mM)</td>
<td>%</td>
</tr>
<tr>
<td>Z-Arg-Arg-MNA</td>
<td>18.4</td>
</tr>
<tr>
<td>Z-Arg-Arg-MNA</td>
<td>8.5</td>
</tr>
<tr>
<td>Z-Arg-Arg-MNA</td>
<td>4.6</td>
</tr>
<tr>
<td>Z-Arg-Arg-MNA</td>
<td>6.2</td>
</tr>
<tr>
<td>Bz-Arg-Arg-MNA</td>
<td>6.3</td>
</tr>
<tr>
<td>D-Val-Leu-Arg-MNA</td>
<td>6.5</td>
</tr>
<tr>
<td>D-Val-Leu-Arg-MNA</td>
<td>6.4</td>
</tr>
<tr>
<td>Gh-Lys-Arg-Pha</td>
<td>50.2</td>
</tr>
<tr>
<td>Gh-Leu-Arg-Pha</td>
<td>16.1</td>
</tr>
<tr>
<td>Gh-Leu-Arg-Pha</td>
<td>1.1</td>
</tr>
<tr>
<td>Guo-Ala-Arg-Pha</td>
<td>1.1</td>
</tr>
<tr>
<td>Guo-Ala-Arg-Pha</td>
<td>1.1</td>
</tr>
<tr>
<td>Bz-Arg-Arg-MNA</td>
<td>0.1</td>
</tr>
<tr>
<td>Bz-Arg-Arg-MNA</td>
<td>0.1</td>
</tr>
<tr>
<td>Arg-Arg-MNA</td>
<td>0.5</td>
</tr>
<tr>
<td>Arg-MNA</td>
<td>0.5</td>
</tr>
<tr>
<td>Ala-Ala-Arg-MNA</td>
<td>0.5</td>
</tr>
<tr>
<td>Lys-Pha</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Samples of 3 µg purified enzyme were used for assays.

Table II

Inhibitor Sensitivity of the Purified Enzyme

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Final Conc.</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFP</td>
<td>10 µM</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>PMSF</td>
<td>10 µM</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>N-ethylmaleimide</td>
<td>5 mM</td>
<td>75 ± 30</td>
</tr>
<tr>
<td>DTNB</td>
<td>5 µM</td>
<td>100 ± 99</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>50 µg/ml</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>50 µg/ml</td>
<td>20 ± 40</td>
</tr>
<tr>
<td>Thiostatin</td>
<td>50 µg/ml</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>50 µg/ml</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Hemin</td>
<td>50 µl</td>
<td>25 ± 55</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mM</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Samples of 3 µg purified enzyme were used for assays.

Table III

Levels of High Molecular Weight Proteases in Various Tissues and Cells of Rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>log_{10}(g tissue)</th>
<th>log_{10}(mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.5 ± 0.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.4 ± 0.7</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.6 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Lung</td>
<td>1.7 ± 0.6</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.6 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.8 ± 1.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.5 ± 0.5</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Muscles</td>
<td>2.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Colcemid</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Extensor Calf Muscle</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>2.0 ± 0.6</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Skin</td>
<td>2.0 ± 0.6</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

Table IV

Inhibitor Sensitivity of the Purified Enzyme

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Final Conc.</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFP</td>
<td>10 µM</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>PMSF</td>
<td>10 µM</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>N-ethylmaleimide</td>
<td>5 mM</td>
<td>75 ± 30</td>
</tr>
<tr>
<td>DTNB</td>
<td>5 µM</td>
<td>100 ± 99</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>50 µg/ml</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>50 µg/ml</td>
<td>20 ± 40</td>
</tr>
<tr>
<td>Thiostatin</td>
<td>50 µg/ml</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>50 µg/ml</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Hemin</td>
<td>50 µl</td>
<td>25 ± 55</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mM</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Samples of 3 µg purified enzyme were used for assays.

Table V

Subcellular Localization of High Molecular Weight Protease in Rat Liver

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>Protein (mg)</th>
<th>Enzyme Content (µg/mg protein)</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear/Plasma Membranes</td>
<td>350</td>
<td>0.75 ± 0.2</td>
<td>95</td>
</tr>
<tr>
<td>Heavy Mitochondria</td>
<td>240</td>
<td>0.25 ± 1.1</td>
<td>45</td>
</tr>
<tr>
<td>Light Mitochondria/ LysoSomes</td>
<td>54</td>
<td>0.22 ± 0.5</td>
<td>55</td>
</tr>
<tr>
<td>Microsomes</td>
<td>135</td>
<td>7.81 ± 14.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Cytosol</td>
<td>487</td>
<td>19.92 ± 83.4</td>
<td>83.4</td>
</tr>
</tbody>
</table>

Subcellular fractions were prepared as described. Experimental Procedures were as described from 1 g of rat liver. Each fraction was disrupted by freeze-thawing three times before assay.